Review

Augmentative biological control: research and methods to help make it work

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Abstract

Augmentative biological control, especially in field situations, can be complex but there are novel or incompletely explored directions for research and methods development that may lead to improved future performance. At the fundamental level, augmentation faces inherent ecological/ behavioural challenges such as enemy dispersal, pest refugia and deleterious interactions within predator/parasitoid guilds. However, these may be addressed by the choice of natural enemies with specific or manipulatable dispersal capabilities which attack hosts when most vulnerable. Integration of augmentative biological control with other control methods has not been sufficiently explored. One promising partner is the sterile insect technique (SIT) and related technologies such as conditional-lethality and genetic-drives that perform best at low and declining pest densities and should interact well, even synergistically, with natural enemy augmentation. Other candidates for integration with augmentative biological control include the addition of plants that support natural enemies, infochemicals such as kairomones that could lead pests to occupy more vulnerable microhabitats and even certain pesticides. At the production level, new or not widely adopted technologies for mass-rearing can lower costs. For example, host irradiation simplifies the handling of parasitoids, improves sanitation, facilitates the movement of natural enemies across borders and allows hosts to be exposed in sentinel-traps. Parasitoid rearing expenses could be halved by using thelytokous strains such as those resulting from *Wolbachia* infections. In some cases, hosts for specialist parasitoids can be obtained inexpensively from the unwanted sex of mass-reared pests: another advantage of integration with SIT. While many innovations are now costly, scientifically sophisticated and planned for use in regional-scale projects, new techniques and genetic modifications could become widely available to the agricultural industry.

Populations of natural enemies grow with those of their prey, but are often a step behind, reproduce more slowly or even fail to maintain themselves locally because of seasonal absence of hosts/food in agricultural environments [1]. As a result, pests can reach economically damaging levels before they are located by predators or parasitoids or these become sufficiently abundant to suppress herbivore populations [2]. Early augmentation can reintroduce enemies to an area or give them an opportunity to dampen the initial growth phase of herbivore numbers (inoculative release). If natural enemies are continually released, they maintain artificially

high predation levels that force incipient pest populations to still lower levels (augmentative release *sensu stricto*).

Thus, to repeatedly release appropriate numbers of effective natural enemies at proper places and times, when the equivalent is unlikely to occur naturally, is the essence of augmentative biological control. This sounds deceptively simple. In practice, particularly in the field situations addressed here, it can be complex and there are many ways, and combinations of ways, for augmentation to fail. An oft-discussed survey concluded that augmented natural enemies were able to manage field pests economically in only ~20% of well-controlled experiments [3, 4] and that failures were the result of a dozen causes as diverse as pest-refugia and predator-cannibalism.

Yet, augmentation in the field has its enthusiastic supporters. Even conservative estimates find it effective, if not necessarily economic, at least 1/5 of the time and there is a highly experienced cadre of practitioners who believe it is practical in many other cases that have been as yet difficult to experimentally confirm [5–8]. Recent thorough field studies have followed augmentation from the costs of natural enemies to positive profits from the crop [9], and a public understanding of the environmental consequences of insecticide alternatives may raise the cost-threshold for use of biological control (e.g. pesticide taxes [10, 11]).

The following is by no means an exhaustive review of augmentative biological control, its problems and potentials; there are a number of excellent, more comprehensive reviews relevant to natural enemy augmentation e.g., Cônsoli et al. [12], Gurr and Kvedaras [13], Reynolds [14] and Williams [15]. Rather, it is an attempt to highlight what seem to be novel or incompletely explored directions for research and methods development. These are organized as follows: (1) ecological challenges that augmentative biological control can face and tactics used to confront them; (2) integration of augmentation with other forms of control; and (3) new (or not widely adopted) techniques that promise to improve natural enemy mass-production and hence lower the costs of augmentation.

Ecological Challenges and Possible Solutions

Collier and Van Steenwyk [3] discuss why many augmentation experiments have failed to find significant pest suppression. Several of the more difficult to address ecological/environmental reasons are revisited in order to appreciate in a better manner what sort of research might be needed to advance augmentative biological control.

Enemy dispersal and pest immigration

Once released, natural enemies may deliberately move away from the target area for a number of reasons including low host densities and high levels of competition [16]. A classic case of unwanted dispersal stems from the non-reproductive physiological state of aggregated, over-wintering coccinellids. When field-harvested and then released, they typically disperse from target sites within 24 h [17, 18]. This movement may be mitigated by the use of flightless morphs [19, 20]. In *Trichogramma* spp., propensity to disperse can be estimated beforehand by examination of wing size [21] and perhaps shape [22]. Natural enemy behaviour may be modified to minimize dispersal [23]. Continuous exposure of the ichneumonid *Mastrus ridibundis* (Gravenhorst) to its codling moth (*Cydia pommonella* [L.]) host prior to release results in a ~40-fold decrease in its dispersal rate [24] while dispersal of the aphid parasitoid *Aphelinus asychis* Walker is positively related to the size of the released cohort [25].

Numerous and diverse plants emit volatiles when under attack that then attract natural enemies (herbivore-induced plant volatiles (HIPV)) [26]. Enemies could be held in target sites by embedding synthetic versions of the plant's 'call for help' in the field [27] or physiologically/genetically modifying crop/companion plants to increase the volume/specificity of the signal [28]. As in any relatively new technology there are large numbers of unknowns and potential problems whose solutions will ultimately determine its future usefulness [29]. However, two of these concerns might be mitigated in the case of augmentative biological control: (1) Hyperparasitoids and parasitoids of predators also respond to HIPVs [30, 31]. Evolutionary relationships between these fourth trophic level responders and plants are unclear, but *Brassica oleracea* L. under attack by *Pieris rapae* (L.) (Pieridae) caterpillars already parasitized by the braconid *Cotesia glomerata* (L.) are induced to emit approximately twice the amount of an HIPV compound attractive to hyperparasitoids as do those being eaten by unparasitized larvae [31]. When natural enemies are augmented they would probably swamp populations of their own enemies and if the released insects seasonally disappear then build-ups of 4th level consumers might return to their original pre-release levels. (2) Pulling parasitoids or predators into a specific location could denude the surrounding region of natural enemies: 'robbing Peter to pay Paul' [32]. With augmentation substantial numbers of new enemies are added locally and these might eventually disperse to raise mortality over an even greater area.

Other plant-based products and green leaf volatiles that presumably act as arrestants, increase parasitism by *Trichogramma* spp. [33] although not all field trials have been successful [34]. Extracts from kairomones, such as lepidopteran eggs and scales, have also produced mixed results in the field [34, 35]. Once arrested, there is a danger that uniformly dispersed host-cues will confuse foraging natural enemies and prevent them from locating prey that act as point sources of kairomones. Sticky-glandular and hooked plant trichomes entrap insects and hold their carrion where it attracts and maintains

a number of hemipteran and spider predators [36]. Perhaps the spreading of zoo-foods such as the corpses of easily reared *Drosophila* spp. might be useful for holding mass-released predators in a target site.

Assuming that beneficial insects will diffuse from a target site into adjacent habitat, the size of the area to be treated will influence how easily a suitable natural enemy density can be maintained. That is because the relative length of a field's perimeter increases at a lower rate than its area (for a square, $4n$ versus n^2), so that the boundary across which augmented natural enemies may wander and/or pests from outside continue to immigrate is relatively greater in a smaller target site [37]. How large an area should be treated and at what release rate are often difficult problems to resolve [38]. One means of addressing both issues might be to test different enemy densities in various sized plots. The plot size at which a negative linear relationship between natural enemy numbers and plant damage appears would be the minimum area that needs to be treated. That is, at smaller plot sizes immigration and emigration would confound the effect of release rate and make it a poor predictor of crop loss. Release rates could then be estimated from that minimum-area relationship of enemy density with herbivory. If no relationship occurs at the largest practical plot sizes then augmentation may not be an economic solution to that particular pest problem. Such otherwise laborious experiments might be simplified by the use of Response Surface experimental designs and statistical methods [39].

Pest refugia

Asymptotic dose response curves are common in augmentative control and this can be attributed to some proportion of pests occupying refugia that place them beyond the reach of particular natural enemies [40]. These protected pests invalidate the assumption that continually higher natural release rates will result in continually higher pest mortality. As an example of pest-refugia, tephritid fruit fly larvae in larger fruit are more difficult for their opiine braconid parasitoids to reach with their ovipositors [41]. When releases are made into environments with large fruit, such as commercially-grown species bred for unusual size, it would be best to augment larval-prepupal parasitoids with longer ovipositors, or better yet egg-prepupal species that forage for relatively shallowly-placed eggs [42]. Refugia can change over time as well as among habitats. Lettuce aphids, *Nasonovia ribisnigri* (Mosely), eventually move to inhabit the tightly packed leaves at the heart of more mature Romaine lettuce. Once there, predators and parasitoids have limited access [43]. Natural enemies, such as certain Syrphidae that are adept at exploiting the early-season infestations that still occur in low densities on relatively open leaves have the best chance of exerting control [44].

Deleterious natural enemy interactions

Intraguild predation in terms of biological control is the consumption of natural enemies of a target pest by their fellow target pest predators and parasitoids [45]. For instance, generalist predaceous beetles that consume both aphids and aphid parasitoids or facultative hyperparasitoids can result in decreased capacity by parasitoids to exert control [46]. If the intraguild predator with broader appetite does not subsequently consume more of the target pest than would have its intraguild victims, then overall control will decline [47]. Mutual interference is where the behaviour of one natural enemy influences that of another, often to leave the host patch. Interference can be both intra- and interspecific and result in asymptotic mortality relationships between enemy and prey densities [48]. There are characteristics that help suggest which combinations of natural enemies are more or less likely to engage in intraguild predation [46]. However, these may not be easy to predict *a priori* [49, 50] and mutual interference can be an issue in the absence of predation. For example, when enemies forage for the same prey in the same manner and at the same time they are functionally redundant and adding species to the guild should increase its diversity but leave prey suppression unaffected. However, combined augmentation of the relatively similar *Trichogramma ostriniae* Pang et Chen and *T. nubilale* Ertle and Davis resulted in lower European corn borer, *Ostrina nubilalis* Hüber, egg parasitism than either species released individually, probably as a result of mutual interference [51].

Biological control can affect natural enemy diversity and host/prey diversity the efficacy of biological control. Augmentative releases could have long-term effects on natural enemy guild structure, e.g. if the mass-release of a superior intrinsic competitor eliminates an inferior [52]. On the other hand, prey diversity can generate apparent competitions, the asymmetric effect of shared natural enemies on multiple hosts, which go on to provide substantial additional pest suppression. The addition of a rapidly reproducing host could result in larger than previous numbers of a parasitoid that also attacks the more slowly reproducing original target [53]. Prey diversity can provide a diverse predator diet and improve nutrition and survival [54]. However, all the above may more likely influence population dynamics over multi-generational time. They are not peculiar to, and perhaps not characteristic of, periodic inundations by a possibly ephemeral natural enemy.

Opportunities for Integrated Augmentative Biological Control

Integration of augmentative biological control with other control methods has not been sufficiently explored in many agricultural systems [40]. A recent review

addressed the integration of biological control in general with sterile insect technique (SIT), host plant resistance, induced plant defences (previously discussed) and cultural techniques, all of which, and others, have applicability to augmentation in particular [14].

Sterile insect technique (SIT)

The SIT, and similar technologies where modified pests are reared and then released into the field, has entered into a Renaissance [55, 56]. New sexing strains eliminate females early in their development and will dramatically cut costs and improve efficacy [57], conditional lethalities where the offspring of released insects perish outside the permissive mass-rearing environment remove the need for damaging irradiation to induce sterility [58], the capacity to genetically manipulate insect endosymbiotic bacteria, *Wolbachia* and others, will foster development of cytoplasmic-incompatibilities, an alternative means of sterilization [59], and also the basis of genetic-drive systems to force desired genes into pest populations [60].

Most of these, including genetic drives, work better as pest densities fall and so would benefit from combination with a suppression-technique that is effective at high pest densities, e.g., augmentative biological control [60, 61]. The felicity of this relationship, with its possibility of synergistic control [62, 63], has long been recognized in SIT programmes, particularly those that provide barriers to the movement of tephritid fruit flies and fruit infesting Lepidoptera across national borders or into fly-free/low-prevalence agricultural zones [64–66].

Increased plant diversity

Even augmented parasitoids and predators [67], not expected to survive indefinitely or continue to maintain their populations after releases stop, may require food and shelter, and could benefit from having alternative hosts/prey [54]. Landscape modifications, often the addition of flowering plants to agroenvironments to provide floral/extrafloral nectar for adult parasitoids can concentrate and conserve natural enemies [68–70] and may also sustain those that are periodically introduced *en masse*. In one case, additions of flowers to vineyards did little to potentiate and conserve augmented *Trichogramma* [71]. However, for the most part, what plant recourses are necessary and how much would be adequate for a large influx of parasitoids remains to be determined. Nectar plants are mimicked to a degree by sugar foods and while there is substantial evidence that sugar solution sprays increase endemic natural enemy abundance and diversity, if not crop-profits [72], an attempt to enhance the efficacy of mass-released *Trichogramma* spp. with sugar was unsuccessful [73]. Better results were obtained by simply feeding parasitoids honey prior to release [73–75].

Besides food and shelter, added-plants can concentrate hosts/prey and perhaps multiply the numbers of natural enemies. Trap plants, for instance, those that receive pests in 'push-pull systems', could be specific targets for natural enemy augmentation, particularly if the hosts/prey are relatively vulnerable once they are repelled from the crop and attracted to the trap [76, 77]. Added banker-plants that harbour alternative prey/hosts early in the season might multiply argumentatively released natural enemies in time for them to attack the target pest in the primary crop [78]. Periodic additions of crop plants previously inoculated with predators or parasitoids can be a particularly effective manner of augmentative release [79].

Lesser appreciated infochemicals

Avoiding the perceived risks presented by natural enemies is energetically costly, physiologically stressful, results in lost feeding and reproductive opportunities and can have similar effects on mortality and population growth as direct predation [80, 81]. Pisurid spiders with functional mouthparts and those glued shut inflicted similar mortalities on *Melanoplus femurrubrum* De Geer grasshoppers [82], and up to 80% of the effect of damsel bug (*Nabis* spp.: Nabidae) predation on pea aphid, *Acyrthosiphon pisum* Harris, population growth is owing to behavioural responses of prey to the risks predators pose rather than direct predation [83]. The tension between eating and being eaten creates a 'landscape of fear' that influences when, where and for how long herbivores will choose to feed, e.g., spiders with and without functional mouthparts caused similar movements of grasshoppers from grasses to structurally more complex and sheltering herbs [84].

Could fear be used to enhance augmentative biological control? For example, to concentrate pests or cause them to move to microhabitats where they are more vulnerable to the mass-released natural enemy. An instance of the desired effect can be found in pea aphids which drop to the ground in the presence of coccenelid predators where they are vulnerable to Carabidae. The resulting mortality rates are nearly double than additive predation by the two beetles species alone [85, 86]. Cues suggesting the presence of a predator might be as effective at eliciting risk-related behaviours as the predator itself. The sight of even dead predators causes Lepidoptera and Hymenoptera to avoid flowers [87] and spider silk alone deters herbivory by both Mexican bean beetles (*Epilachna varivestes* Muisant) and Japanese beetles (*Popillia japonica* Newman) [88]. There is an indication that volatile chemicals are involved in the latter effect which raises the possibility of their synthesis and application as a stressor and goad to movement. It might even be possible to genetically transform plants to produce predator-cues. When pests have alarm pheromones, as in aphids, these can elicit

similar behaviours as direct evidence of a natural enemy and they have been genetically inserted into a transformed wheat strain ([89, 90]; however, see [91]).

Pesticides

Broad spectrum pesticides that kill or damage natural enemies are obviously not conducive to simultaneous augmentative biological control and are generally seen as an impediment to progress by beneficial insect producers. Even organic-certified insecticides generally considered to be benign can have detrimental effects on predators, parasitoids and pollinators [92]. A recent and graphic example of the indirect costs of pesticides is the improved biological control of aphids in various Chinese crops following the wide-spread adoption of Bt varieties and the resulting decrease in sprays [93].

However, insecticides can be integrated into certain forms of augmentation and pesticides can sometimes have minimal effects on important natural enemies. For example, larval *Trichogramma* spp. are often sheltered in their host-eggs from toxins and their augmentative releases have been successfully integrated into insecticide treatments for *O. nubilalis* [9]. Some pesticides are extremely benign. Since *Trichogramma* have a single larval instar and larvae do not moult they are unaffected by ecdysone agonist tebufenozide [94]. Alternatively, a strain of *Trichogramma chilonis* Ishii has been developed whose adults are resistant to endosulfan insecticides [95]. Protein + insecticide 'bait-sprays' are applied as droplets to suppress tephritid fruit flies. The braconid parasitoids of these flies are not attracted to the bait and are relatively unaffected by the spinosad toxicant allowing augmentative releases to be combined with pesticide treatments [96]. However, even bait sprays can have unintended consequences [97] and certain classes of non-targets have attracted little concern, e.g., Lepidoptera larvae and other large herbivores that consume droplets they are not necessarily attracted to.

Techniques for Mass-Rearing and Release

Irradiation

Removal or sterilization of unparasitized hosts has a number of benefits for mass-rearing and augmentative biological control. For example, tephritid fruit fly larvae are routinely irradiated prior to their exposure to braconid parasitoids [98, 99]. Since factory parasitism typically ranges from 50 to 90% large numbers of pests could be included in parasitoid cohorts destined for release. However, at the proper radiation dosage, adult hosts fail to develop, but parasitism is unaffected or even improved [100, 101]. This much simplifies the handling and distribution of the parasitoids, improves factory sanitation,

facilitates the movement of natural enemies across borders and quarantine barriers and allows hosts to be exposed in sentinel-traps without the danger of their escape and reinfesting treated areas.

Host irradiation can also preserve hosts for long periods until they are required for parasitoid rearing; e.g., calypterate fly pupae used in production of idiobiont ectoparasitoids [102, 103]. Radiation slows the development of *Cotesia flavipes* Cameron, a braconid parasitoid of larval Lepidoptera. With the addition of cool temperatures this allows the storage of pupae and can defer emergence by about 30 days [104].

Modified parasitoid sex ratios

There can be considerable economic advantages to mass-rearing and dispersing all-female (thelytokous) strains/species of parasitoids [105]. Only females generally contribute to pest mortality and some costs of augmentation could be cut in half by not investing in the production of males. Thelytokous hymenopteran populations are not uncommon in nature and are often the result of infection by intracellular endosymbiotic bacteria, particularly various strains of *Wolbachia pipientis* [106]. Unfertilized eggs destined to be males are transformed into diploid females by either prevention of chromosome reduction during meiosis or through postmeiotic fusion of two haploid mitotic products [107].

To be practical, all-female strains must meet two assumptions: (1) arrhenotokous (haplo-diploid bi-sexuality) and thelytokous females must have at least similar abilities to locate and oviposit into hosts and (2) thelytokous populations have a greater capacity for increase under mass-production conditions. *Wolbachia* infections can induce effects other than sex-ratio distortions and infected individuals might be inferior biological control agents. Infected *Aphytis melinus* DeBach (Aphelinidae) suffer decreased fecundity and longevity [108]. A comparison of arrhenotokous and thelytokous lines of *Trichogramma cordubensis* Vargas and Cabello and *T. deion* Pinto and Oatman found that arrhenotokous insects, created through the application of antibiotics to thelytokous females, had higher fecundity and dispersed further in laboratory tests [109]. However, there were no differences in dispersal in greenhouses, and the absence of males made thelytokous wasps preferable. Seemingly minor differences between all-female and bisexual strains have been identified in foraging behaviours of figitids [110]. Attack rate, but not host-handling time, was unaffected by *Wolbachia* infection in *Trichogramma brassicae* Westwood [111] and the bacteria had no effect on fecundity in *T. atopovirilia* Oatman and Platner [112]. *Wolbachia* infection may even be beneficial. Certain *Wolbachia* are capable of increasing the fecundity of *Drosophila melanogaster* Meigen hosts by influencing iron utilization in restricted or overloaded diets [113], while others

increase the fecundity and lifespan of *Aedes* spp. hosts [114, 115].

The second assumption underlying the proposed superiority of thelytokous mass-rearing cultures is that the absence of males lowers production costs per female. As noted above, some strains of *Wolbachia* increase lifespan in *D. melanogaster*, but this effect can disappear when males and females are placed together, as they would be under mass-rearing conditions [116]. If the symptoms of infected parasitoids are similarly density-dependent, this could influence population growth rates and even eliminate the advantage of all-female rearing colonies.

Two challenges face the adoption of *Wolbachia* induced thelytoky, the accumulation of deleterious alleles in the absence of sexual reproduction [117] and how such strains are to be obtained in the first place. Although artificial horizontal transfers of *Wolbachia* through micro-injections of infected tissue into novel hosts are possible [118], they have proven difficult and the infections are often transient [119]. Alternatively, thelytokous strains could be obtained by: (1) systematically searching for all-female populations guided by evolutionary theory for evolution and maintenance of sex. One effort at this has failed [120]; (2) attempt horizontal transfers through super- (or multi-) parasitism, a means by which infections may be transmitted in nature [121, 122] and (3) genetically modify *Wolbachia* already present in the wasp to produce sex-ratio distortions.

Female perceptions of host quality can also be manipulated to yield more female-biased sex ratios. Since size has a greater influence on the fitness of parasitoid daughters than on sons, ovipositing females that can estimate host size tend to lay diploid-female eggs in larger hosts [123]. By gradually increasing the sizes of leafminer hosts exposed to the eulophid *Diglyphus isaea* (Walker) the % male progeny decreased from 58 to 48% [124]. Simply mixing large and small hosts had a similar effect on sex ratio and resulted in females that had the same biological control-quality as those reared under the standard all-large-hosts procedure [125].

Host acquisition

The use of more easily reared factitious hosts can be a double-edged sword. On the positive side, cost savings in mass-rearing can make augmentation feasible. For instance, the ability to rear *Trichogramma* spp. on the eggs of grain feeding moths, thus eliminating the expense of maintaining plants for target pest egg-stock, allowed further development of egg-parasitoid augmentative control [126]. The negative side is that a natural enemy reared on a factitious host is by its nature not a specialist and there is the possibility of non-target effects and loss of focus on control aims. This is not always an issue, for instance, there may be few vulnerable non-targets in the release sites [127], and in some cases hosts for specialist parasitoids can be obtained from the unwanted sex of mass-reared pests [128]. For example, the sexually dimorphic development period of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Tephritidae), allows mature female larvae to be easily separated from males destined for SIT releases. Female larvae can then be used as hosts for argumentatively-released braconids such as *Diachasmimorpha longicaudata* (Ashmead). These larvae are rarely completely parasitized and in a further step, the pupae from larvae exposed to larval parasitoids can be re-exposed to pupal parasitoids if the latter do not successfully superparasitize the primary parasitoid [129, 130]. *In vitro* rearing has the potential to reduce augmentative biological costs, but even the relatively successful case of *Trichogramma* faces a number of challenges when scaled up to mass-production levels, such as local lack of automation and sanitation [131].

Conclusions

If and when the efficacy of augmentation becomes further established, research is likely to flow towards such an elegant and environmentally friendly technique. From the perspective of one who has worked on tephritid fruit fly augmentative biological control, a multi-scientist, long-term, multi-government-funded project, the scientific future of such relatively well financed undertakings that address regional and international agricultural problems looks particularly promising. However, the scale and expense of this research and its implementation is considerably beyond what many would consider the typical practice of natural enemy augmentation. For example, in order to produce and apply the up to 50 million parasitoids a week Mexico releases in support of fly-free and low-fly-prevalence agricultural zones, the *Mosca Fruta* programme has access to the production of a tephritid mass-rearing factory employing scores of workers, an irradiator for host-treatment and aircraft for delivery. Some of the other research and method developments discussed require genetic manipulations, quarantine facilities and sophisticated chemical analyses that might be difficult for a small-scale insectary business to duplicate.

On a positive entrepreneurial note, many of these techniques and genetic modifications could become increasingly available, their production privatized and then sold to the state. Analogous to the US space programme, there will be technical spin-offs and industry can sometimes use expensive government equipment such as a particle beam accelerator for sterilization (e.g., Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida, USA).

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